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Bioremediation Potentials of *TRICHODERMA HARZIANUM* and *GLOMUS MOSSEAE* on the growth of *CAPSICUM ANNUM* L. grown on soil irrigated with water from mining site

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ABSTRACT
In this research, the effects of *Trichoderma harzianum* and *Glomus mosseae;* singly and in combination on *C. annum* irrigated with mining water was investigated. The quality of mining water used for irrigation of *Capsicum annum* and the irrigated *Capsicum annum* samples were collected and analysed for their heavy metal build-up. The results indicate that the mining water used was contaminated with Copper (19.6 mg/l), Arsenic (15.2 mg/l), Lead (6.54 mg/l) and Iron (45.8 mg/l) respectively. It was observed that the two organisms were able to utilize the heavy metal constituents, with *G. mosseae* alone showing high efficiency of utilization. Dual inoculations were also observed to be better heavy metals utilization than the control. The research concluded that the use of *Trichoderma harzianum* and *Glomus mosseae* on polluted soil improves growth and yield of *C. annum* in contaminated soils.

Keywords: *Trichoderma harzianum, Glomus mosseae, Capsicum annum,* Mining water, Heavy metals.

INTRODUCTION

Human needs are viewed as a means of meeting the needs of the current generation without compromising the ability of the future generation as well [1]. One of the resources needed to achieve this human need is water because most livelihood activities depend on the availability of water. Increasing activities of human beings such as mineral exploration threaten the water sources on which we all depend. According to the Environmental Mining Council of British Columbia [2], water has been called “mining’s most common casualty”. Water and soil contamination caused by acid mine drainage (AMD) is a significant environmental problem in most parts of the world, particularly in densely populated developing countries where human habitats are usually in close proximity to mine sites [3]. Water draining from mining site frequently contains heavy metals at high levels which could contaminate streams and agricultural lands when used for irrigation purposes. In West Africa and particularly Nigeria, urban agriculture has been a normal practice along various river banks. These river/streams have been reported to be highly contaminated by toxic chemicals from mining site and industries which discharge untreated wastewater into receiving waters. The level of heavy metal contamination in soils from farm irrigated with water from rivers is not adequately articulated [4].

Many techniques of soil remediation have been developed, such as physical, chemical and photo-degradation. However, biological treatment offers the best environmental friendly method for remediating heavy metal-contaminated soil because it utilizes the capability of the indigenous microorganisms in the soil environment to break down the metals into innocuous substances [5]. Biological remediation, a process defined as the use of microorganisms such as *Glomus mosseae*, *Trichoderma harzianum* and *Pseudomonas aeruginosa* to detoxify organic and inorganic xenobiotic compounds from the environment [6].

Glomus mosseae which is an arbuscular mycorrhiza fungus is used for the treatment of polluted soils [7]. It is obligate biotrophs that live symbiotically in the roots of the majority

of vascular plants such as pepper. It helps plants in the uptake of water and nutrients such as phosphorus and micronutrients from the soil [8] and in turn, the plant provides fixed carbon to the fungal partner.

Trichoderma harzianum is an active rhizosphere colonizer and this fungus produces antibiotics such as gliotoxin, viridin, cell wall degrading enzymes and also biologically active heat-stable metabolites such as ethyl acetate [9]. It as many positive effects on plants such as increased growth and yield, increased nutrient uptake, increased percentage and rate of seed germination and induced systemic resistance to plant diseases. *Trichoderma* harzianum strains play an important role in the bioremediation of contaminated soil with pesticides and herbicides. They have the ability to transform a wide range of insecticides such as organochlorines, organophosphates and carbonates [10] [11].

Peppers are vegetable crops belonging to the family of *Solanaceae* and genus *Capsicum*, which are native to tropical America and Africa. In Nigeria, pepper is mainly grown around the savanna ecological zones in a mixed cropping or as a mono crop both during the rainy season and dry season under irrigation. Species commonly cultivated are *Capsicum annum* known as sweet pepper, bell pepper, and cherry pepper. Sweet peppers commonly known as "Tattase" in Nigeria are non-pungent with walled fruits. Pepper is good source of vitamins A, C, E, B1 and B2 potassium, phosphors and calcium [12]. However, the estimated yields of 9 t/ha obtained on farmers' fields from Nigeria are often very low, compared with the estimated yields of 15 t/ha obtained in Western Europe [13]. The low yields obtained in Nigeria have been attributed to a number of production constraints that includes problems of diseases, pests, poor irrigation management and environmental pollution which result in low soil fertility [14]. This present research examined the bioremediation potentials of *T. harzianum* and *G. mosseae* in remediating polluted soils using *C. annum* as test plant.

METHODOLOGY

The research was conducted in the screenhouse of Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. Contaminated water from mining site was obtained from tourmaline mining site, Ifewara Road, Ile-ife, Osun State. The water was collected into sterile kegs. Seeds of sweet pepper, some mycorrhizal inoculum containing spores of *Glomus mosseae* and a culture *Trichoderma harzianum* were obtained from Mycology unit of Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. A sieved mixture of topsoil and river sand in the ratio of 10:1 was used for the propagation of *Glomus mosseae* after the mixture had been steam sterilized by heating for three hours at 131^{o}C and left to cool for four days. The inoculum was propagated using *Zea mays* for a period of fourteen [14] weeks. After fourteen weeks of growth, the soil with the roots (cut into pieces) mixed together was harvested and stored in jute bags and taken to the laboratory for spore assessment.

The spore of *Trichoderma harzianum* was sub-cultured using 39 g potato dextrose agar which is a growth media for fungi, dissolved in 100 ml of distilled water. The mixture was homogenized and autoclaved as appropriate after which, it was brought out to cool before been poured into sterile petri dishes and allowed to solidify. The *Trichoderma harzianum* was aseptically inoculated into the petri dishes and incubated at 37^{O}C for 7 days. Spores were harvested from agar plates by flooding with sterile distilled water. The spore solution was standardized using a haemocytometer to 10^{8}spores/ml. Treatments

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were applied at the point of transplanting the *Capsicum annum* seedlings into experimental pots. Thirty grams (30 g) of *G. mosseae* and 30 ml *T. harzianum* were used for single inoculation while 15 g + 15 ml of *G. mosseae* and *T. harzianum* were used for dual inoculation. Seedlings were left for two weeks to establish from transplanting shock before irrigating the soil with mining water at concentrations of 5 and 10% v/w. Growth parameters of the plant such as plant height, number of leaves, leaf area and stem girth were measured weekly for ten weeks. The data were analyzed using SAS 9.2 statistical software, by subjecting the data to descriptive and inferential statistical methods. For plant growth parameters, means were separated using Fisher's Least Significant Difference (LSD) test. Also, determination of Heavy Metals (Mn, Fe, Pb, Cu, Zn, As) of plants tissue and fruits were analyzed. The plant tissue analysis was done by weighing half grams of the plant`s leave, oven dried and crushed. The crushed samples were placed in a platinum crucible and transferred into a murffle furnace and ashing was done at 600°C for three hours, subsequently removed and allowed to cool in a desiccator. Five milligrams of 6N HCl was added to the ashed samples and left for 30 minutes to be properly digested. Filtration was done using Whatman No. 1 filter paper into a 50 ml conical flask and the filtrate made up to the 50 ml mark with distilled water. Shaking was done vigorously and the heavy metals (Mn, Fe, Pb, Cu, Zn and As) were read with the aid of Atomic Absorption Spectrophotometer (AAS).

Table 1: Analysis of Variance showing Mean Squares for Growth Parameter across the weeks at 5% Concentration of Contaminated Mining Water

Source	Degree of Freedom	Number of Leaves	Plant Height (cm)	Leaf Area(cm²)	Stem Girth (cm)
Reps	2	14.03	2.59	86.62	0.03
Treatment	3	477.49**	3694.79**	157537.79**	1.26**
Week	9	1032.68**	4098.09**	181763.02**	8.32**
Treatment x Week	27	38.15*	120.58**	10004.61**	0.18**
Error	198	17.31	27.61	548.92	0.04
CV		21.44	16.54	14.30	14.71
R^2		77.47	90.36	95.63	92.28

Table 2: Analysis of Variance showing Mean Squares for Growth Parameter across the Weeks at 10% Concentration of Contaminated Mining Water

Source	Degree of Freedom	Number of Leaves	Plant Height (cm)	Leaf Area (cm²)	Stem Girth (cm)
Reps	2	0.61	15.62	39.82	0.09
Treatment	3	208.08**	5268.18**	200771.07**	3.06**
Week	9	1245.47**	5269.52**	208128.82**	11.78**
Treatment x week	27	110.82**	267.37**	8866.61**	0.29**
Error	198	24.76	28.55	3096.34	0.05
CV		24.62	16.25	34.31	16.46
R^2		75.15	92.58	81.58	92.54

*, ** mean significant at probability level 0.05

Table 3: Effect of treatments on growth parameter at 5% concentration of contaminated mining water

Treatments	Number Of Leaves	Plant Height(Cm)	Leaves Area (Cm^2)	Stem Girth (Cm)
1-SS+TP	17.97	20.85	86.68	1.05
1w SS+-TP	18.10	23.62	99.560	1.09
2- SS+GM	16.67	29.24	201.60	1.24
2w- SS+GM	16.83	34.50	228.40	1.40
3- SS+TH	20.07	35.46	156.20	1.27
3w- SS+TH	26.47	47.44	202.70	1.56
4- SS+GM+TH	17.93	27.16	154.40	1.14
4w- SS+GM+THW	21.23	35.94	180.80	1.47
LSD	**3.82**	**6.85**	**46.95**	**0.30**

(Mean values at 0.05 level of probability)

Table 4: Effect of treatments on growth parameter at 10% concentration of contaminated mining water

Treatments	Number Of Leaves	Plant Height(Cm)	Leaves Area (Cm^2)	Stem Girth (Cm)
1-SS+TP	17.60	20.89	72.79	1.04
1w SS+-TP	17.80	22.64	84.25	1.04
2- SS+GM	21.03	30.13	114.00	1.37
2w- SS+GM	19.47	36.21	248.10	1.39
3- SS+TH	17.03	38.91	208.50	1.39
3w- SS+TH	27.30	50.43	214.90	1.74
4- SS+GM+TH	21.63	28.04	124.20	1.24
4w- SS+GM+THW	19.83	35.87	230.90	1.66
LSD	**4.43**	**7.99**	**50.68**	**0.37**

Legend
SS: Sterilised soil
TP: Test plant
GM: *Glomus mosseae*
TH: *Trichoderma harzianum*
w: weekly wetting
LSD – Least Significant Difference
Treatment 1 – Sterilised soil + *Capsicum annum*
Treatment 2 – Sterilised soil + *Capsicum annum* + *Glomus mosseae*
Treatment 3 – Sterilised soil + *Capsicum annum* + *Trichoderma harzianum*
Treatment 4 – Sterilised soil + *Capsicum annum* + *Glomus mosseae* + *T. harzianum*

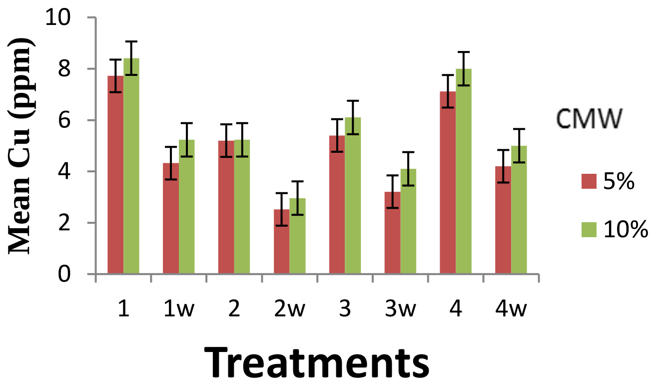

Fig 1a: Heavy metal (Cu) content of plant under different treatments

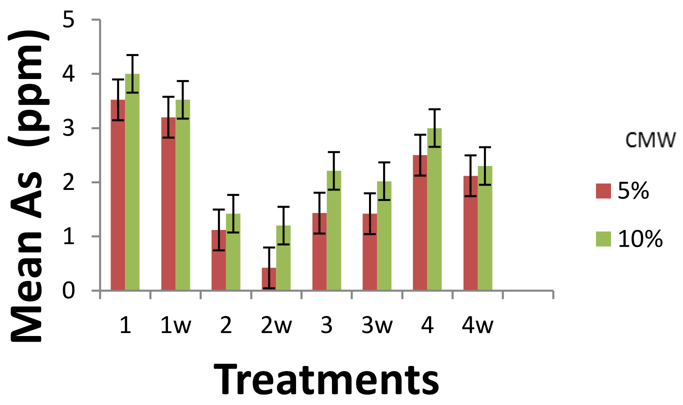

Fig 1b: Heavy metal (As) content of plant under different treatments

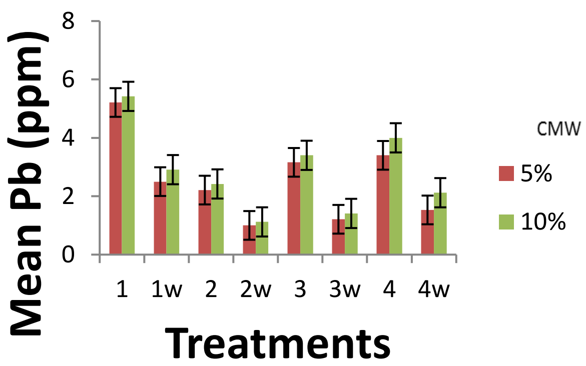

Fig 1c: Heavy metal (Pb) content of plant under different treatments

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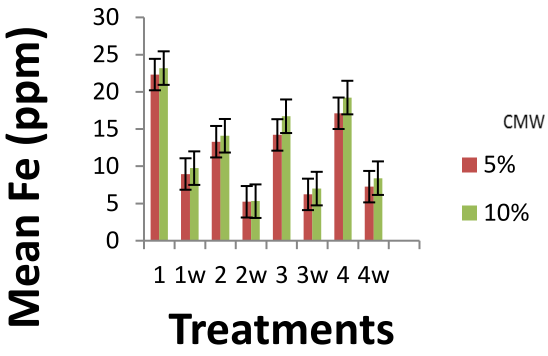

Fig 1d: Heavy metal (Fe) content of plant under different treatments

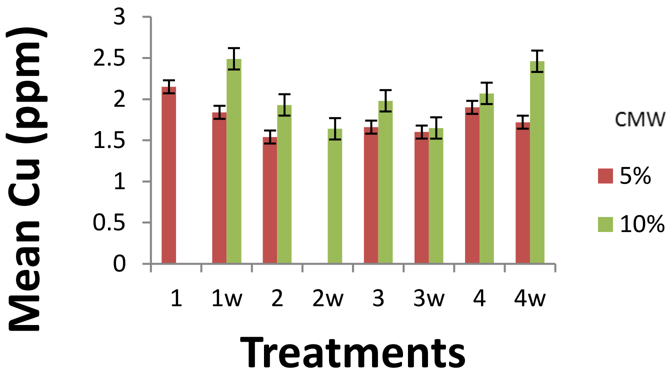

Fig 2a: Heavy Metal (Cu) Content of Fruits under Different Treatments

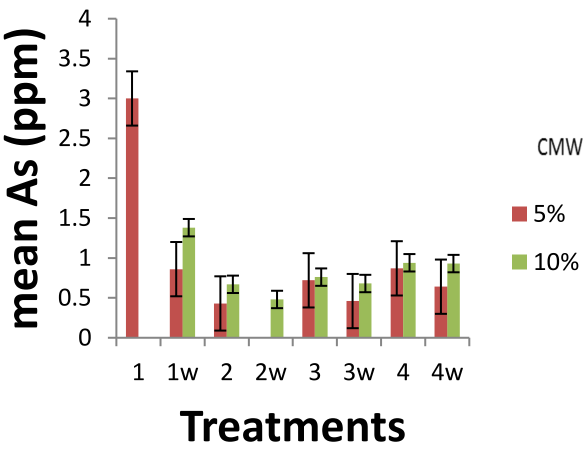

Fig 2b: Heavy Metal (As) Content of Fruits under Different Treatments

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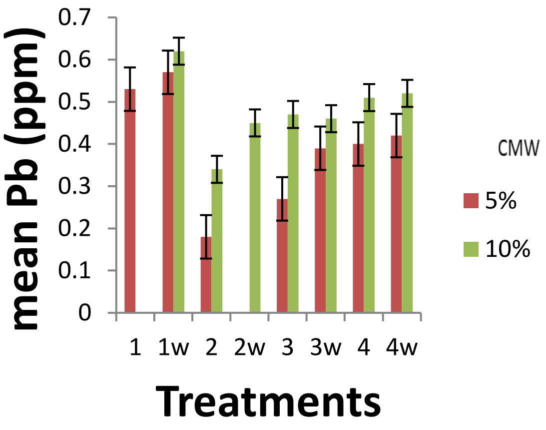

Fig 2c: Heavy Metal (Pb) Content of Fruits under Different Treatments

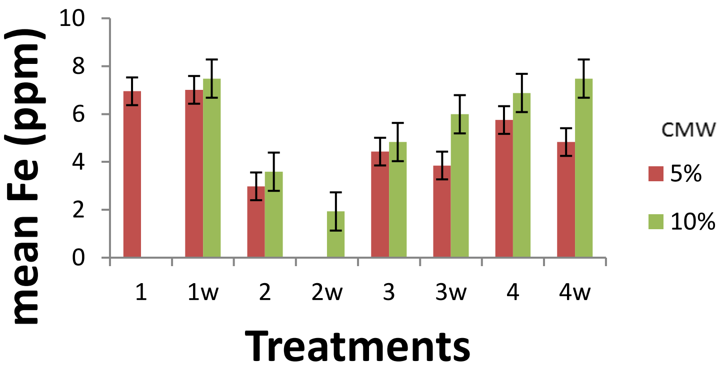

Fig 2d: Heavy Metal (Fe) Content of Fruits under Different Treatments

Legend

1-SS + TP
1w-SS + TP
2-SS + GM
2W-SS + GM
3-SS + TH
3W-SS + TH
4-SS + GM + TH
4W-SS + GM + TH

W– Weekly wetting of plants with contaminated mining water
GM – *G. mosseae*
TH – *T. harzianum*
TP - Test Plant
CMW- Contaminated Mining Water
Error Bar- 95 Confidence level

RESULTS AND DISCUSSION

The effect of mining water polluted soil at 5% and 10% under the influence of *Glomus mosseae* and *Trichoderma harzianum* on the growth response of *Capsicum annum* was shown in the Analysis of Variance from Table 1 and 2, it indicated that the replicates, weeks after planting, treatments and treatment by weeks after planting values were highly significant across the number of leaves, plant height, leaf area and stem girth. The R-square for all the growth parameters shows that the model used in the data analysis is reliable. Also, the coefficients of variance were low which means that the variables analyzed was closely related and there is no much disparity among the variables and the variables analyzed is reliable.

The effects of *G. mosseae* and *T. harzianum* on the test plant irrigated with mining water was shown in Table 3 and 4, *Capsicum annum* seedlings exhibited various degrees of responses to the level of stress imposed by mining water and its enhancement with *T.*

harzianum and/or *G. mosseae*. These changes were as a result of physiological stress caused by the effect of the different treatments used in this research. *Capsicum annum* grew best on soils inoculated singly with *T. harzianum* and *G. mosseae* compared to those irrigated with mining water without treatments. This can be related to the effect of *Trichoderma* on growth promotion as a result of the elimination of minor pathogens or deleterious microorganisms in the rhizosphere, thus allowing the plants to reach their maximal developmental potential [15] and also ability of arbuscular mycorrhiza fungus which helps plants in the uptake of water and nutrients such as phosphorus and micronutrients from the soil [8]. Also, treatment with dual inoculation of *Trichoderma harzianum* and *Glomus mosseae* performed better than treatments without inoculation of microorganisms. This result implies the synergistic relationship between the two organisms. It was observed from this research that the values of the growth parameters decreased as the contaminated mining water concentration increased. This is due to changes in the soil chemical condition as a result of the increased concentration of contaminated mining water and it is similar to the findings of [16] and [17]. This effect can also be attributed to the fact that higher concentrations heavy metals such as As, Mn, Pb strongly poisons the metabolic activities of plant [18].

The results showed the bioaccumulation of all the heavy metals analyzed in the plant on Figure 1a, b, c and d; and on figure 2a, b, c and d for fruits, it was revealed that as the concentration of the mining water increased, the heavy metal accumulation also increased across all the treatments. Treatments inoculated with *G. mosseae* alone had lowest content of heavy metals across all the concentration level and did better than other treatments. This can related to the findings of [7] that arbuscular mycorrhiza fungi (*G. mosseae*) can play an important role in alleviating heavy metal phototoxicity.

CONCLUSION

This research concluded that water from tourmaline site is potential source of heavy metals in crops grown around it but the inoculation of *T. harzianum* and *G. mosseae* as treatments to polluted soil was effective for bio-transformation and increased plant growth.

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